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Original article

Control of Genetic Purity of Parental Lines of Sunflower by Allelic Variants of Electrophoretic Spectra of Seed Storage Proteins

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Abstract

The efficiency of sunflower hybrid breeding is determined by the genetic purity of the parent lines. Traditionally, plant selection in the process of seed production of the obtained lines is performed by morphological traits. This does not always ensure high typicality of the lines. Evaluation of plants of sunflower lines only by morphological traits is not always effective, and in some cases leads to a decrease in the typicality level of both lines and hybrid combinations. The studies have established and identified allelic variants of electrophoretic spectra of seed storage proteins. Simultaneous evaluation and selection of plants by electrophoretic spectra of storage proteins and by morphological traits of plants ensured an increase and preservation of the genetic homogeneity of the maternal line GE106. The using of allelic variants of electrophoretic spectra of proteins in plant selection ensured the genetic purity of the sterile analog $CYT^{S}rfrf$ (genotype has sterile cytoplasm, genes for restoration of polly fertility in homozygous recessive state) of the line at the level of 98.7%, the fertile analog $CYT^{N}rfrf$ of the line at the level of 98.6% (method of soil control). With this selection method, the analysis of plants by allelic variants of protein spectra increased the indicators of genetic purity of the maternal line components. The level of genetic purity of the sterile and fertile analogue, determined by allelic variants of the protein spectra of seeds, in the sterile analog $CYT^{S}rfrf$ and the fixer of sterility $CYT^{N}rfrf$ of the line was 99.6% and 99.8%. Establishing typicality by protein spectra in some cases helps to increase the indicators of genetic purity of lines, since it does not allow identifying atypical plants by height.

Keywords: Sunflower, Genetic Purity, Morphological Traits, Spectra of Proteins, Allelic Variants of Spectra

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INTRODUCTION

In the breeding of sunflower hybrids, the manifestation of the heterosis effect in hybrid combinations is largely determined by both the selection of parental lines for hybridization and their genetic homogeneity (Aksyonov, 2014; Kostevich and Medvedeva, 2018). In the process of creating lines, selecting plants by typicality, distinctive morphological features are used. Determination and description of morphological traits of plants for identification of breeding material is an important condition for effective breeding and seed production of parental lines and sunflower hybrids with high genetic purity (Aksyonov, 2005; Gonzalez-Perez et al., 2004). Evaluation and selection of plants by morphological traits allows to control the typicality of parental lines and hybrids, to reject atypical plants that appear during uncontrolled mixing of seeds of different genotypes, during uncontrolled cross-pollination. These factors lead to a significant decrease in the indicators of varietal properties of lines and hybrids (Hafiz Ghulam Muhu-Din Ahmed et al., 2022).

However, the using of plant evaluation only by morphological traits in the process of creating the breeding material and in seed production may not always be effective and may reduce the real indicators of the level of typicality of sunflower lines and hybrids. Identification of different genotypes only by morphological traits (by phenotype) may have less reliable and incorrect character This is explained by the hidden genetic variability of genotypes. The evaluation of plant in the process of selection by morphological traits may be incorrect, since different morphotypes of plant are the result of the same gene mutation (pleiotropic effect of genes), and the same morphotypes plant may be the result of changes in different genes, which leads to genetic contamination of seed material (Sabetta et al., 2011; Suvorova, 2019; Zeinalzadeh-Tabrizi et al., 2018). A new mutation in a gene, pleiotropy of a gene can fully influence the manifestation of traits that are associated with the action of this gene in the genotype. The pleiotropy effect may cause problems in selective selection of sunflower plants in the process of line development and evaluation, when one allele of a gene dominates during selection for one trait and another allele of the same gene dominates during selection for other traits (Bachlava et al., 2010; Demurin et al., 2010).

To determine the typicality and prevent genetic contamination of sunflower breeding and seed material, accessible and more reliable methods of plant evaluation and selection are needed in the process of creating parental lines and their reproduction in seed production. The introduction of molecular genetic methods into the practice of breeding work gives breeders new opportunities for studying and identifying breeding and seed material. The using of the method of electrophoresis of storage proteins of sunflower seeds solves the problem of more reliable control over the genetic purity of genotypes. With this method of analysis and control, allelic variants of electrophoretic spectra of storage proteins are used as a systematizing trait of the genetic purity of parental lines and sunflower hybrids (Nikolić et al., 2014). The obtained information about sunflower genotypes by allelic variants

of components of electrophoretic spectra of seed storage proteins allows more reliable control of genetic purity, preservation and support of a high level of typicality. The main criterion for using seed storage proteins in sunflower breeding and seed production is specific and identified protein spectra of each genotype, which do not depend on the conditions of cultivation and seed storage. The method of identification at the biochemical level of sunflower lines and hybrids obtained as a result of crossing parental lines allows to increase the accuracy of plant selection and ensures a more complete manifestation of the heterosis effect in crossing combinations (Gavrilova and Anisimova, 2003; Iqbal et al., 2011; Loskutov et al., 1994)..

Thus, the purpose of the presented scientific work is to study the possibilities of using allelic variants of electrophoretic spectra of storage proteins of sunflower seeds in the implementation of individual selection in breeding and seed production of plants of parental sunflower lines to increase and maintain genetic purity.

MATERIALS AND METHODS

The research was conducted at the Department of Biology and Agronomy of Luhansk National University (Starobelsk, Mirgorod, Ukraine) during 2020-2023. The object of the study was morphological traits and allelic variants of electrophoretic spectra of storage proteins of seeds of the maternal sunflower line GE106.

The evaluation and selection of plants of the line were carried out according to morphological traits during the vegetation period and according to allelic variants of electrophoretic spectra of storage proteins of seeds. Allelic variants of electrophoretic spectra were established by the method of electrophoresis of storage proteins. Seeds of the sterile analogue $CYT^{S}rfrf$ and fertile analogue $CYT^{N}rfrf$ of line GE106 were selected for analysis and identification of protein spectra using the method of proteins electrophoresis.

In the laboratory conditions, each selected seed of the line was individually numbered and each numbered seed was cut in half. The halves of the seeds without the embryo were subjected to the method of electrophoresis of storage proteins. Each numbered seed without the embryo was analyzed separately by comparing the allelic variants of the protein spectra on the resulting electropherogram.

The second halves of the seeds with the embryo were identified and arranged in groups according to the allelic variants of the electrophoretic spectra of helianthinins in accordance with the individual numbers.

The halves of the seeds with the embryo, having allelic variants of the protein spectra that are characteristic and typical for line GE106, and in accordance with the individual numbers of the analyzed halves of the seeds without the embryo, were selected in a separate variant 1 for sowing in field conditions as typical. Also, for sowing in field conditions, numbered halves of seeds with

embryos and with protein spectra identified as typical and atypical for line GE106, but with the same morphological features characteristic of plants of this line, were selected in separate variant 2 (this was the actual varietal purity of the line according to morphological characteristics). The selected halves of seeds with embryos were planted in pots with soil according to variants to obtain seedlings. In the phase of 1-2 pairs of true leaves of the plant line were planted in open ground.

The selected and sown plants of the variant 2 were subjected to different selection and evaluation methods in subsequent years and were divided into three variants.

Variant 1: plants were selected only by the method of evaluation by morphological traits (control).

Variant 2: plants were selected by electrophoretic spectra of seed storage proteins and simultaneously by morphological traits of plants in field conditions.

Varint 3: at the first reproduction and selection - by electrophoretic spectra of seed storage proteins, then only by morphological traits of plants.

During the flowering period, the plants of the sterile analogue were cross-pollinated with pollen from the fertile analogue.

The typicality of the plants of the sterile and fertile analogues for each variant was determined after harvesting by electrophoresis of storage proteins in laboratory conditions and the following year by the morphological characteristics of the plants.

Electrophoresis of storage proteins of seeds was carried out according to the method of F.A. Popereli. According to the method, the seed kernel was freed from the husk. Each individual extracted seed kernel was crushed to a homogeneous state. The crushed seed kernels were placed in a solution of glacial acetic acid with acetone to remove fat. The solution for removing fat had a ratio of 30 ml of glacial acetic acid per 1.0 l of acetone. The crushed seeds in the solution were mixed. After mechanical mixing, the solution in the test tube was placed in a thermostat for 20 minutes at a temperature of 31.0 C. After 20 minutes, the solution in the test tube was again mechanically mixed and centrifuged for 10 minutes at 5000 revolutions of the centrifuge per minute. After centrifugation, the degreasing solution (glacial acetic acid + acetone + vegetable fats of the seed kernel) was removed from the test tube using a water-jet pump. The sunflower seed storage proteins (helianthinins) remained in the test tube. Helianthinins were extracted in an acidic medium consisting of distilled water, glacial acetic acid, and urea (1 l of solution contained 30 ml of glacial acetic acid and 120 g of urea). The extraction solution with seed helianthinins was placed in a thermostat to settle. After settling, the solution was centrifuged again and prepared for electrophoresis. Electrophoresis was performed in an acidic polyacrylamide gel. The gel consisted of acrylamide, glacial acetic acid, methylene bis-acrylamide, and urea. The electrode buffer (pH 3.1) contained glycine and glacial acetic

acid. Electrophoresis was performed at a voltage of 500 V and an initial current of 50 Ma per plate of the electrophoretic chamber. Electrophoresis of the storage proteins lasted for 2.5-3.0 hours. The helianthinin strips of seeds on gel plates were fixed and stained in a solution containing glacial acetic acid, trichloroacetic acid, acetone, and Coomassie brilliant blue R-250 paint powder. All solutions for conducting helianthinin electrophoresis were prepared in distilled water using pure chemical reagents. After staining, the helium plates were washed with tap water and ethyl alcohol. The description of the electrophoretic spectra of proteins was performed based on the localization and intensity of protein components in the electrophoretic spectra on the electropherogram. A level of genetic purity of plant was determined on the basis of typical and atypical spectra of proteins on the electropherogram.

The evalution of plants by morphological traits was carried out the following year in the field using the field control method. In each soil control plot, 500 seeds of each studied variant were sown with an inter-row width of 70 cm. The repetition of the variants was twofold.

The results of this research were analyzed by MSTAT test, and means were compared by Tukey's multiple comparison test at 5% level.

RESULTS AND DISCUSSION

The conducted analysis of electrophoregrams of different sunflower genotypes allowed to establish allelic variants of protein spectra. Alleles of genes responsible for polypeptide synthesis are presented on electrophoregrams by bands. The established genes of genotypes were named after the name of seed storage proteins - Heliantinins. Hybridological analysis of hybrid combinations allowed to establish 10 genes and their allelic variants on electrophoregrams - *HEL 1, HEL 2, Hel 3, Hel 4, Hel 5, HEL 6, HEL 7, HEL 8, HEL 9, NK* (Figure 1).

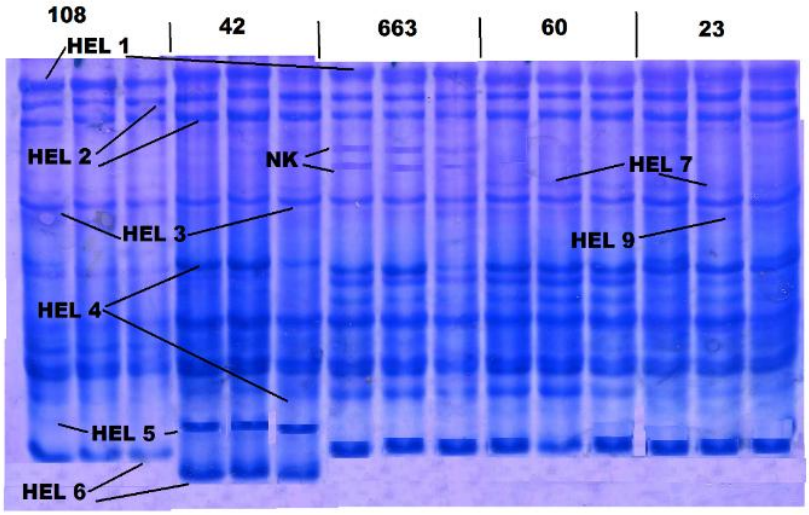

Figure 1. Gene loci encoding helianthinin in self-pollinated samples of sunflower.

Heliantinins of the fourth protein in different genotypes are represented by a set of homologous polypeptides in different variants and they are encoded by the locus of the HEL4 gene. The established allelic variants resolved the issue of identification and determination of genetic purity of breeding and seed material.

A higher efficiency of evaluation and selection plants of sunflower line GE106 based on allelic variants of protein spectra was noted when using electrophoretic spectra of storage proteins with the main components of the protein fraction, 11S globulins, as a systematizing feature.

Electrophoresis of seed proteins and comparison of allelic variants of protein spectra allowed us to establish the initial actual low level of typicality of the sterile analogue of the line of 44.4% and the sterility fixer of the line of 49.2%. The level of typicality of the line, established by the morphological characteristics of plants, was significantly higher and amounted to 87.4% for the sterile analogue and 87.0% for the sterility fixer.

The using of plant selection by allelic variants of electrophoretic spectra of protein with simultaneous evaluation of plants by morphological traitsin field conditions allowed to significantly increase the level of typicality of lines. With such evaluation and selection of plants, typicality, determined by field control methods, the sterile analogue was 98.7%, and the pregnancy fixer was 98.6% (Table 1).

Table 1. The typicality level of the maternal line of sunflower GE106 under different methods of selection and evaluation of plant, %, 2020-2023.

Method of selection and evaluation	Analog of line	Atypical plants		Level: 1	Level: 2
		by height	by morphological traits		
variant 1	Ast*	4.7±0.33	7.7±0.41	87.6	92.3
	B_{fx}	4.6±0.29	8.1±0.38	89.5	91.9
variant 2	Ast	0.9±0.05	0.4±0.05	98.7	99.6
	B_{fx}	1.2±0.09	0.2±0.05	98.6	99.8
variant 3	Ast	4.7±0.26	2.6±0.17	92.7	97.4
	B_{fx}	4.1±0.37	3.5±0.24	92.4	96.5
$LCD_{0.05}$		1.2	1.1	0.4	1.0

All data differences are proven at the level of $LSD_{0.05}$.
Indicate significant differences at $P < 0.05$ by ANOVA-analyze. comparison of options is carried out based on the difference in the indicators of the trait.

*Note: Ast – sterile analog CYT^{S}rfrf;
B_{fx} – sterility fixer CYT^{N}rfrf.
Level: 1 – Level of typicality by morphological traits.
Level: 2 – Level of typicality by allelic variants of protein spectra.

With this method, all protein spectra after simultaneous selection and evaluation of plants by allelic variants of electrophoretic spectra and morphological traits have the highest level of typicality among the studied variants of the research 99.6% (sterile analog), 99.8% (sterility fixer). The selection

and evaluation of plants at the first stage by allelic variants of electrophoretic spectra, then only by morphological characteristics in field conditions led to a decrease in the line typicality indicators by the field control method for the sterile analogue to 92.7% for the sterility fixer to 92.4%. When evaluating plants of the line using the field control method, the lowest level of typicality analogues of line GE106 of 87.6 and 89.5% was observed when selection and evaluation of plants only by morphological traits.

The typicality indices of line analogues for all variants were higher when determining genetic purity by electrophoresis. The typicality of line analogues determined by the method of allelic variants of protein spectra was higher in the first variant by 4.7-2.4%, in the second variant by 0.9-1.2%, in the third variant by 4.7-4.1% than with the field control method based on morphological traits. Allelic variants of electrophoretic spectra of helianthinins do not identify the sunflower plants that are atypical in height (tall plant, low plant). These plants are identified as typical by protein spectra using electrophoresis, which leads to overestimation of typicality indices. The lowest number of atypical plants of line analogues by the height trait of 0.9-12% was achieved during the evaluation and selection of plants simultaneously by allelic variants of electrophoretic spectra of helianthinins and morphological traits in field conditions.

The study, analysis of inheritance and variability of electrophoretic spectra of sunflower shows that intraspecific polymorphism of globulins is caused by the manifestation of allelism of helianthin-coding loci (Anisimova et al., 1991). This solves the problem of the main approaches to the using of storage proteins as marker traits for identifying genotypes, for constructing phylogenetic schemes, for solving problems of breeding, plants selection, seed production and seed control. The results of a comprehensive study of protein seeds and the polypeptide composition of helianthinin demonstrated that the degree of difference and similarity of seed storage proteins can serve as a measure of control over the genetic purity of r genotypes (Strigina et al., 2005; Pomorova et al. 2019).

Our analysis of the components of protein spectra of electrophoresis confirms this position that the genetic control of helianthinins is carried out by a maximum of 9-10 loci. Self-pollinated sunflower samples and lines are characterized by the constant presence of the loci *Hel 1, HEL 2, Hel 3, HEL 4, HEL 6* in the genotypes. The loci *Hel 5, Hel 7, Hel 8, Hel 9, NK* are found in individual lines created as a result of interspecific hybridization. Hybridological analysis of parental components, plants of the first and second generations show that the allelic variants of the genes *HEL 2, Hel 3, Hel 8* do not change and are always stable. The loci *Hel 5, Hel 7, Hel 9, NK* were characterized by the trait: manifestation-absence (Figure 1).

The decrease in the typicality of the sterile CYT^{S}rfrf and fertile CYT^{N}rfrf analogues of the line was due to the appearance in the genotypes of allelic variants of the *Hel 1, Hel 4, Hel 6* loci that are atypical for the line GE106. A clear manifestation of heterozygosity was noted for the *Hel 1, Hel 6*

loci on the electropherograms. Such a manifestation of heterozygosity is unacceptable for the genotypes of homozygous parental lines. Namely, individual selection of the sterile analogue plant and the sterility fixer by allelic variants of the electrophoretic spectra of proteins with simultaneous control by morphological features of plants and subsequent determination of genetic purity by electrophoresis and soil control methods ensured an increase in the typicality of the line during the study cycle.

Uncontrolled selection based on protein spectra or its complete absence results in a significant reduction in the genetic purity of seed material (Kirichenko, 2005; Konarev A. A. et al., 2000). The manifestation of atypical allelic variants of the components of the protein spectra of the studied line in the experiment is due to the presence by sterile analog $CYT^{S}rfrf$ of a heterozygote for the first locus *Hel 1*, the first component of the fourth locus *Hel 4*, a heterozygote *Hel 1* (first and second components) and the second component of the sixth locus *Hel 6* (Figure 2).

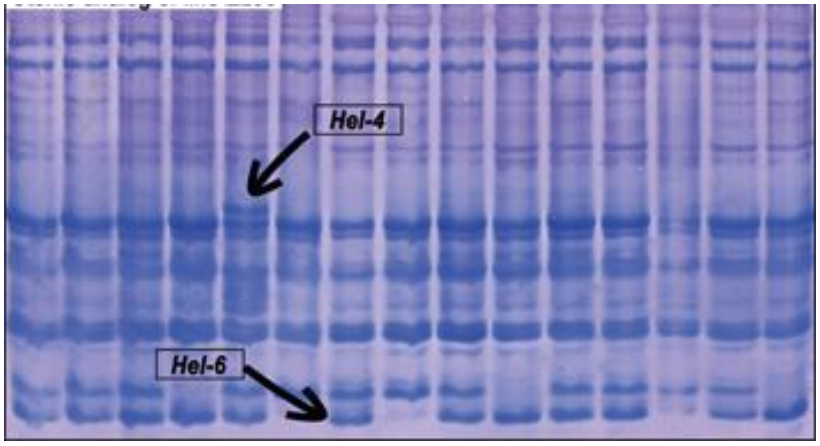

Figure 2. Allelic variants of electrophoretic spectra of storage proteins of seeds of sterile analogue of sunflower line after selection by morphological traits.

When selecting based on morphological characteristics, electrophoresis of storage proteins shows the appearance of atypical protein spectra at the *HEL 6* locus (Figure 3).

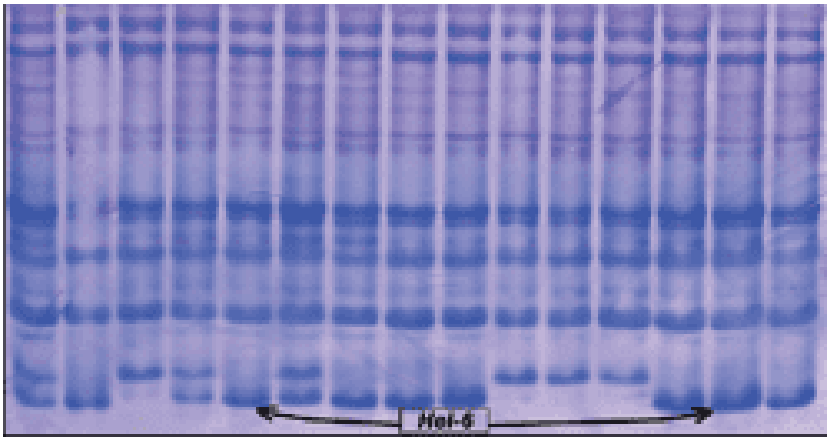

Figure 3. Allelic variants of electrophoretic spectra of storage proteins of seeds of sterile analogue of sunflower line after selection by morphological traits.

The presence of non-culled and non-removed plants with atypical protein spectra during selection by morphological traits shows a high genetic contamination of the line and significantly reduces the level of its typicality, determined by electrophoresis and soil control (Figure 4).

Figure 4. The plant of the maternal line GE106 after selection of only morphological traits

At the monolocus level, homozygotes and heterozygotes are distinguished on the basis of allelic variants of protein spectra, whereas morphological mutations are often recessive, less often dominant, therefore, it is usually impossible to distinguish a heterozygote from both homozygotes when conducting soil control, which ultimately causes the appearance of atypical plants and a decrease in genetic purity (Chesnokov, 2019).

As shown by the research of scientists, the identified allelic variability of the composition of helianthinin polypeptides in sunflower genotypes and the codominant nature of inheritance of this trait serve as a theoretical justification for the use of helianthinin as a genetic marker for identifying genotypes, determining the genetic purity of inbred lines and monitoring the hybridity of seeds during interlinear hybridization (Allen et al., 1987; Anisimova et al., 1991).

This approach in application in our experience of an electrophoresis of seed proteins and comparison of allelic variants of protein spectra allowed us to establish a really low level of typicality of the sterile analogue and sterility fixer - 47.4 and 49.3% at the initial determination of the typicality of the line. The decrease in the genetic purity of the line is due to the appearance of allelic variants of the *Hel 1, Hel 4, Hel 6* loci that are atypical for it.

Analysis of electrophoretic differences (allelic forms) reveals genotypes with new atypical components in the protein spectrum that are not observed in the electropherogram of line GE106. Thus, the electrophoresis method allows us to identify recombinants with new atypical protein spectra that cannot be predicted because they are not formed as a result of a simple combination of protein spectra and these genotypes are not established by the morphological characteristics of plants (Borodulina and Suprunova, 1977).

The presence of non-culled and non-removed plants with atypical protein spectra during selection based on morphological characteristics shows high genetic contamination of the line GE106 and significantly reduces the level of its typicality when it is determined by allelic variants of protein spectra using electrophoresis methods.

The manifestation of heterozygosity was noted for the genes *Hel 1*, *Hel 6*, which is completely unacceptable in breeding and seed production for homozygous inbred lines. In order to increase the typicality of the line during the research cycle, individual selection of a sterile analogue and sterility fixer was carried out according to the morphological characteristics of plants (the first variant) and according to allelic variants of electrophoretic spectra of proteins with simultaneous control by morphological characteristics of plants with subsequent determination of genetic purity by electrophoresis and soil control methods.

The most effective method for increasing and maintaining the line at a high level of genetic purity is selection of plants by electrophoretic spectra of storage proteins with simultaneous control and selection by morphological characteristics. The method of individual selection of plants by protein spectra solves the problem of identifying and culling (removing) plants that do not differ from the line plants by morphological characteristics, but have atypical allelic variants at the *Hel 1*, *Hel 4*, *Hel 6* loci.

This selection method allows achieving the highest level of typicality. The level of typicality, determined by allelic variants of components of electrophoretic spectra of seed storage proteins, is the highest. All protein spectra after simultaneous selections by protein spectra and morphological features are typical (Figure 5).

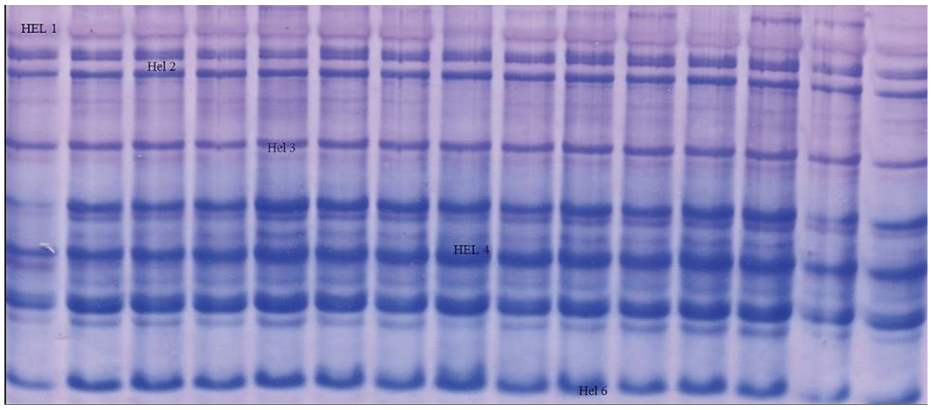

Figure 5. Allelic variants of electrophoretic spectra of storage proteins of seeds of the parental line of sunflower after selection by allelic variants of electrophoretic spectra of storage proteins of seeds and morphological features of plants. All electrophoretic spectra of proteins are typical.

This method of selection and control of genetic purity by allelic variants of electrophoretic spectra allows for the cultivation of plant lines that are identical in morphological characteristics (Figure 6).

Figure 6. The crops of the maternal line of sunflower of Ge106 after selection of plants on protein spectra and morphological traits

At the same time, with such careful selection, when control is carried out both by electrophoretic spectra and by morphological features, the sterile analogue shows the appearance of atypical plants that differ in height. This is probably due to the small number of electrophoretic spectra of storage proteins (five-six) in the line, by which selection and control of plants is carried out at the molecular level by protein markers (Konarev A. V. et al., 2000).

With this method of plant selection and control over genetic purity, differences in proteins in electrophoretic mobility, caused by allelic substitutions in the structural part of the gene, will allow us to establish changes in the genotypic composition of the plant population, to reject atypical plants, and to maintain high typicality of genotypes (Konarev V. G., 1983; Anisimova, 1993; Konstantinova, 2003).

Selection of plants at the initial stage by protein markers, and further selection and control during the reproduction period – only by morphological characteristics of plants, also ensures a high level of typicality of line analogues. However, in the absence of control over the typicality of the line by protein spectra, with further reproduction of the line, the level of typicality, which is determined by the soil control method, decreases.

CONCLUSIONS

The analysis of experimental data shows that the selection of plants by allelic variants of the electrophoretic spectra of seed storage proteins inherent in the analyzed sunflower line GE106, simultaneously with the assessment of plants by morphological characteristics, provides a significant increase in the typicality of the line.

Allelic variability of the composition of protein spectra of helianthinins in seeds can serve as a theoretical basis for the use of this trait for identification and establishment of genetic purity of parental lines in sunflower breeding and seed production.

The level of typicality determined by the method of soil control by morphological characteristics of the line, during its reproduction and selection of plants by allelic variants of the electrophoretic spectra of seed storage proteins and by morphological characteristics reaches 98.7% for the sterile analogue, 98.6% for the fertile one, and by the method of electrophoresis of seed storage proteins it is 99.6 and 99.8%, respectively, for both analogues of line GE106.

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Conflicts of Interest

The author declares no conflicts of interest.

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